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Review

SUMO modulation of protein aggregation and degradation

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Abstract: Small ubiquitin-like modifier (SUMO) conjugation and binding to target proteins regulate a wide variety of cellular pathways. The functional aspects of SUMOylation include changes in protein-protein interactions, intracellular trafficking as well as protein aggregation and degradation. SUMO has also been linked to specialized cellular pathways such as neuronal development and synaptic transmission. In addition, SUMOylation is associated with neurological diseases associated with abnormal protein accumulations. SUMOylation of the amyloid and tau proteins involved in Alzheimer's disease and other tauopathies may contribute to changes in protein solubility and proteolytic processing. Similar events have been reported for α-synuclein aggregates found in Parkinson's disease, polyglutamine disorders such as Huntington's disease as well as protein aggregates found in amyotrophic lateral sclerosis (ALS). This review provides a detailed overview of the impact SUMOylation has on the etiology and pathology of these related neurological diseases.

Keywords: SUMO; neurodegeneration; Alzheimer's disease; Parkinson's disease; Amyotrophic lateral sclerosis; Huntington's disease; tau; amyloid; synuclein; superoxide dismutase; TAR DNA-binding protein-43

1. The SUMOylation pathway

SUMOylation is a process by which polypeptides called small ubiquitin-related modifiers (SUMOs), are covalently linked to lysine residues of cellular target proteins. Mammalian cells express three major paralogues, SUMO1, SUMO2 and SUMO3. The latter two SUMO subtypes are similar to each other in structure, and are commonly referred to as SUMO2/3. The process of SUMOylation is similar to ubiquitination and involves activating (SUMO-E1), conjugating (SUMO-E2) and SUMO-E3 enzymes (Figure 1). The SUMO proteins are initially cleaved at their C-terminus by Sentrin-specific proteases (SENP) to expose a diglycine (GG) motif. The mature SUMO is activated in an ATP-dependent pathway to a complex of SUMO activating enzyme E1 (SAE1) and SAE2 where SUMO is covalently attached via a thioester bond to a cysteine residue in SAE2. The SUMO moiety is subsequently transferred to an active cysteine site within the SUMO conjugating enzyme, Ubc9. The latter enzyme transfers SUMO to a lysine residue in the target protein via a non-covalent complex with a SUMO-E3 ligase of which there are multiple species. The general consensus sequence for SUMO conjugation is ψKxE/D where ψ is bulky hydrophobic residue and x can be any amino acid followed by an acidic residue. The SUMOylation cycle is then completed by the removal of SUMO from its target protein by one of the SENPs [1].

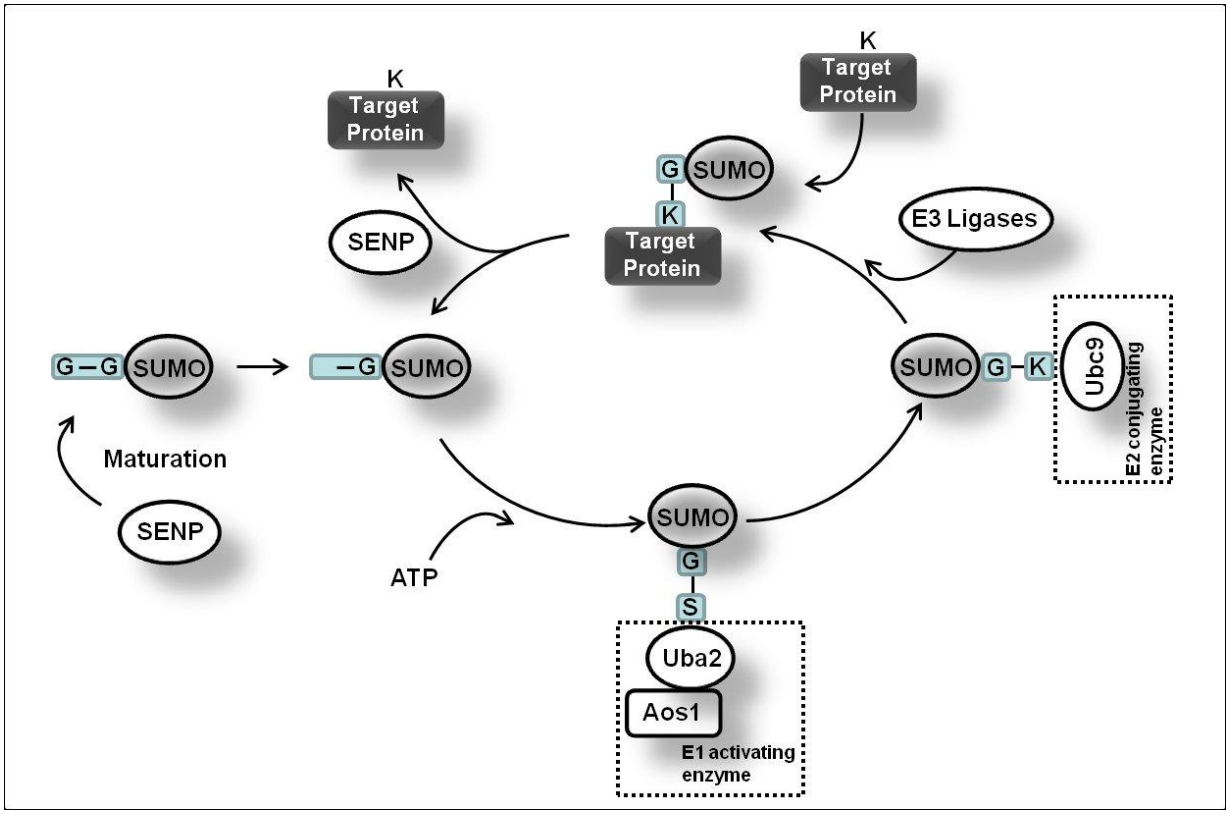

Figure 1. Protein SUMOylation pathway. The cycle begins with the cleavage of SUMO proteins into mature forms by the specific isopeptidases SENPs. The removal of the C-terminal end amino acids of SUMO leads to the activation of the protein by ATP that allows the binding with E1 heterodimer. SUMO is then transferred to the specific E2 enzyme Ubc9 that guides SUMO onto the target protein by recognizing the lysine residue within the SUMOylation motif. E3 enzymes enter into the SUMO cycle to increase the

efficiency of the ligation reaction. SENPs removes SUMO from the target protein by cleaving the isopeptidic bond and SUMO proteins are therefore again able to enter in a second SUMOylation cycle.

The effects of protein SUMOylation vary depending on the target and include altered subcellular localization, activity and stability. SUMOylation may have the effect of revealing or blocking sites for other post-translational modifications such as ubiquitination or phosphorylation. SUMOylation of target proteins can also promote novel protein interactions by non-covalent binding of proteins with SUMO interacting motifs (SIMs). Many proteins with SIMs recognize poly-SUMO chains, which are formed by SUMO2/3 rather than mono-SUMO conjugates formed by SUMO1. Interestingly, several ubiquitin E3 ligases were found to have SIMs suggesting that in some cases protein SUMOylation may help target a protein for degradation (reviewed in [2,3]).

2. SUMOylation and neurodegenerative diseases

SUMO modification of several proteins has been linked to Alzheimer's (AD), tauopathies, and Parkinson's disease (PD) as well as ALS and Huntington's disease (HD). The functional impact of SUMO can involve different pathways related to the production, aggregation and/or clearance of misfolded proteins that are found in these neurodegenerative disorders. SUMOylation has been shown to be involved in the amyloid pathology in AD, potentially by altering the processing and/or trafficking of the amyloid precursor protein, as well as at its downstream level. In addition, conjugation of SUMO1 to the microtubule associated tau protein leads to changes in phosphorylation and subsequent aggregations that may contribute to neurofibrillary tangle (NFT) formation in various tauopathies. Similar effects of SUMOylation have been reported for the huntingtin (Htt) protein and related polyglutamine repeat diseases as well as superoxide dismutase (SOD) and the TAR DNA-binding protein-43 (TDP-43) associated with ALS, although the effect of SUMOylation on TDP-43 is very poorly known. SUMOylation seems to have instead a disaggregation effect on α-synuclein, that is linked to PD, since it abolishes its fibril formation *in vitro*. This review discusses in detail the contributions of SUMOylation to the etiology and pathology of these related neurological diseases.

3. SUMOylation and AD and other tauopathies

AD is a debilitating, progressive neurodegenerative disease, recognized as the most common cause of chronic dementia among the aging population. A progressive impairment in cognitive function due to subtle changes in cellular communication characterizes the onset of the disease, whose pathological progression ends up with a severe synaptic dysfunction leading to significant cognitive deficits in recognition, language and skilled movements [4,5]. Since no effective treatment for AD currently exists, efforts have focused on understanding the molecular mechanisms of the AD-related synaptotoxicity and neuronal loss. The hallmarks recognized for AD are senile plaques, composed primarily of amyloid-β (Aβ) peptides, and NFTs that are the result of accumulations of hyperphosphorylated tau [6,7]. These plaque and tangle pathological features are accompanied by neuroinflammation related to glial cell activation which is considered to be a fundamental contributor to AD pathogenesis [8,9].

Several lines of evidence support the dysregulation of SUMOylation in AD. SUMO2 levels, particularly the high molecular weight region (> 75 kD) (but not SUMO1 or ubiquitin levels), are decreased in the hippocampal formation of AD or other Tauopathies patients [10]. Additionally, an association between a single nucleotide polymorphism (SNP) in genes encoding SUMO enzymes, including Ubc9 and a homolog of SAE2 [11–13] has been found in patients with either sporadic late-onset AD or mild cognitive impairment (MCI) [11].

3.1. SUMOylation and amyloid precursor protein

Several studies have found a potential relationship between APP and SUMOylation. This is noteworthy given that aggregation and accumulation of misfolded proteins is a primary causative factor in AD, particularly as it relates to the Aβ protein production derived from proteolytic process of amyloid precursor protein (APP) [14]. Global changes in SUMO1 and SUMO2/3 conjugation levels are present in the Tg2576 transgenic mouse models of AD [15,16]. Interestingly, APP levels are dramatically increased in the hippocampus, cortex and cerebellum of these mice while elevations of ubiquitinated proteins is mainly observed in the hippocampus [16].

A recent study conducted an age-related analysis of the expression levels of SUMOylated proteins in the Tg2576 mouse [15]. At early stages of the pathology (~ 3 months), significant differences in the SUMOylation patterns were detected, with increases of SUMO1 conjugation at 3 and 6 months of age, but not at 17 months, accompanied by similar patterns of increases for Ubc9 and SENP1. The same study revealed a decrease for SUMO2 conjugation only at later ages (17 months). Consistent with these studies, high molecular weight SUMO2/3 conjugates (> 75 kD) were found to be reduced after 7 months (just prior to the onset of widespread amyloid plaque deposition) in the same animal model, whereas SUMO1 conjugates and Ubc9 were not affected at the late time points [10].

Interestingly, an increase in free SUMO1 but not SUMO2/3 levels was observed in 18-month-old APP transgenic mice [17]. The decrease in SUMO2 conjugation might be due to the presence of toxic Aβ oligomers which have been shown to impair upregulation of SUMO2 (but not SUMO1) conjugated proteins occurring during neuronal activity [10]. Most intriguingly, a significant increase in free SUMO2/3 levels was observed in the hippocampus of 25-month-old C57BL/6 mice compared to 7-month-old mice [18], whereas high molecular weight SUMO2/3 conjugates (> 75 kD) were found to be reduced at 24–26 months [10], suggesting that SUMO dysregulation may play a role in age-related cognitive decline. With regard to this, it is interesting to notice that SUMO1 overexpression impairs synaptic function, spine morphology and memory, suggesting that SUMO1 conjugation might have opposite effect than SUMO2 conjugation [19]. Taken all together, these findings suggest that up-regulation of SUMO2 might be beneficial against the age-related memory decline, as well as AD memory loss. Indeed, supplementing SUMOylation via transduction of its conjugating enzyme, Ubc9, rescues Aβ-induced deficits in LTP and hippocampal-dependent learning and memory [10].

APP is a type 1 transmembrane glycoprotein which was initially identified as a potential SUMO conjugated protein through the use of a proteomic cloning strategy for putative lysine residue(s) that may be modified [20]. Relatively high *in vitro* conjugation efficiency was predicted compared to the values calculated for the *in vivo* modification. It has been shown *in vitro* that APP can be SUMOylated by SUMO1 and SUMO2 at lysines 587 and 595 which are near the sequence for the

β-secretase cleavage [15]. This was supported by the fact that APP constructs with the target lysines substituted conjugation-deficient arginine residues, K587R and K595R, could not be SUMOylated [21]. Replacement of these lysine residues increased the accumulation of Aβ aggregates which suggests that APP-SUMOylation could be a protective mechanism against amyloidogenic APP processing. Consistent with this finding, increasing APP SUMOylation by up-regulating the SUMO-E2 enzyme Ubc9 resulted in a decrease in Aβ aggregate levels [21].

Poly-SUMOylation of APP has been postulated since several molecular weight band shifts have been detected in the western blot. It has therefore been concluded that the conjugation of more than one SUMO chain along the APP protein could be possible. By immunoprecipitating mouse brain extract it was demonstrated that endogenous APP was SUMOylated *in vivo* by both SUMO1 and SUMO2/3 [22]. However, other investigations demonstrated that SUMO2/3 was not co-immunoprecipitated with APP, suggesting the absence of their direct binding. These data were confirmed both in HEK293 and human neuroblastoma cell lines [23]. Interestingly, it underlined a difference in the effects of mono- or poly-SUMOylation on APP amyloidogenesis. By replacing the lysine residue (K11) required for poly-SUMO chains formation with an arginine, the SUMO3 variant obtained was still able of conjugating the monomeric SUMO3 to its target but was unable to form poly-SUMO chains. The co-transfection of HEK293T cells with APP and the SUMO3 variants showed an increase in Aβ generation, indicating that mono-SUMOylation has a reinforcing effect on Aβ production. It was therefore proposed that poly-SUMOylation negatively regulates Aβ production [23].

In contrast, overexpression of APP and SUMO3 in HEK293 cells increased Aβ production. Reducing endogenous SUMOylation by transfecting cells with dominant-negative SUMO3 mutants where one or both of the conserved C-terminal GG-motif was mutated to alanine (AA) residues also increased Aβ suggesting that direct conjugation was not required. Another study suggests that endogenous SUMO may play an indirect role in APP processing and Aβ production. In fact, the knockdown of SUMO induced a decrease in SUMOylated substrates but not in the amyloidogenic processing of APP [24].

Intriguingly, one of the identified lysine targets is also the site of a well-characterized APP mutation (K595N), which is associated with the autosomal dominant Swedish early-onset familial form of AD [25]. It is therefore tempting to speculate that the block of APP SUMOylation at this lysine residue can contribute, at least to some extent, to the pathogenicity and increased Aβ production shown by the Swedish mutation [26]. However, the SUMOylation of APP at K595 *in vivo* and the role of an aberrant SUMOylation in the increase of Aβ levels still have to be determined.

Finally, since a dynamic competition between SUMO proteins stabilization and ubiquitin-mediated proteolysis has been demonstrated, several research groups have examined whether and to what extent APP half-life could be affected by SUMOylation/ubiquitination. In a pulse–chase analysis, even though SUMO3 seemed to alter APP maturation by retarding its glycosylation, the APP half-life was not affected by SUMO1 and -2/3 knockdown, indicating that SUMOylation does not influence Aβ generation by altering the rate of APP turnover [23]. In contrast with these data, another study found a longer APP half-life, due to a 30% decrease in APP degradation after SUMO 3 overexpression in HEK-293 transfected cells [24]. Nevertheless, the surprising finding that inhibition of SUMOylation via SUMO1 and -2/3 knockdown does not affect APP or Aβ levels [24] suggests that endogenous SUMOylation might have indirect, non-covalent modulatory effects on APP processing. Further investigation will be required, taking into consideration other factors such as oxidative stress, the interplay with ubiquitination, eventual

compensatory pathways due to overexpression or knockdown of other SUMO proteins as well as SUMO covalent and non-covalent interactions. Protein SUMOylation has been recognized as an oxidative stress sensor mechanism. It is, in fact, involved in cell death induced by H_2O_2 since it increases JNK phosphorylation during apoptosis [15,27].

3.2. SUMO and tau

The natively unfolded protein tau lacks the typical secondary structure in the absence of a binding partner [28]. It is involved in the regulation of microtubules stability and is responsible for axonal development [29,30]. Under pathological conditions, tau is hyperphosphorylated and accumulates within cells leading to the development of "aggregopathies". The resulting neuronal loss is a defining feature of several neurodegenerative diseases, collectively called "tauopathies". This family of pathologies includes frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Pick's disease (PiD), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) in addition to AD [31,32].

At a molecular level, these diseases are characterized by an abnormal or hyper phosphorylated tau that turns into the peculiar intracellular aggregates known as NFTs [33–35]. The diffusion of tau fibrillation in the brain is measured as Braak stages, currently considered one of the scale indices that determine the severity of the tauopathies during the pathology development [36]. Over 30 tightly regulated phosphorylation sites have been identified within tau and their hyperphosphorylation lies at the basis of the formation of the paired helical filaments seen in AD [37]. Clinical-to-pathological correlation studies have demonstrated that the number of NFTs in the hippocampus and cerebral cortex, consisting of hyperphosphorylated tau, correlates with the actual degree of dementia [38–40]. Tau hyperphosphorylation reduces its ability to bind to tubulin compromising microtubule assembly and stability. Notably, normal tau is sequestered from microtubules and serves as a template for its conversion to a misfolded form and transmission in a "prion-like" manner [41]. In AD, the accumulation of tau aggregates has been confirmed isolating the hyperphosphorylated protein as sedimentable non-fibril cytosolic protein. Indeed such abnormal modifications decreased tau solubility up to 40% [42]. It is interesting that the abnormal phosphorylation of tau is considered a potential biomarker for several neurodegenerative diseases including AD because it is one of the earliest signs of neuronal degeneration preceding tau aggregation. SUMOylation occurs as post-translational modification of tau [43–45]. The microtubule associated protein undergoes SUMO modification at a specific consensus motif VK340SE which is located within the fourth microtubule binding repeat as determined by mutagenesis analysis in which lysine-to-arginine mutants were generated [43]. In a cell-based assay, overexpression of SUMO1 and human tau showed high molecular weight bands corresponding to SUMOylated species. Although at a lesser extent, SUMO2 and SUMO3 also showed similar modifications. These results suggest that tau is not exclusively mono-SUMOylated, but possibly either more than one SUMO molecules conjugate different lysines residues (multiSUMOylation) or the extension of a SUMO-chain could occur on a single lysine (polySUMOylation) [43,44].

Contrasting results have been shown for SUMO modifications of phosphorylated tau. NFTs were found not to be immunoreactive for SUMO1 but were highly positive for ubiquitin immunoreactivity [46]. Additional studies showed that SUMO1 co-localized with the hyperphosphorylated tau in APP transgenic mice [43,45]. Interestingly, examination of APP

transgenic mice demonstrated that SUMO1 co-localizes with phosphorylated tau near neuritic plaques but this was not observed in mutant tau transgenic mice indicating that APP processing can be part of the SUMO-tau interaction [45].

In vitro studies have indicated an interesting interplay between tau SUMOylation and its phosphorylation. It is known that phosphorylation affects the structural and functional protein conformations altering also protein-protein interactions and tau phosphorylation could promote its SUMOylation. Conversely, increased tau SUMOylation results in elevated levels of phosphorylation [47]. Indeed, the expression of SUMO1 and tau in a HEK293T cells line significantly increased tau phosphorylation at Thr-205, Ser-214, Thr-231, Ser-262, Ser-396, and Ser-404 sites. Treatment with different concentrations of ginkgolic acid (GA), a non-specific inhibitor of protein SUMOylation [48], reduced phosphorylation to confirm a dependent relationship. Tau hyperphosphorylation maintained by treating cells with the protein phosphatase 2A (PP2A) inhibitor okadaic acid [49], was crucial to increasing the conjugation by SUMO1 of the hyperphosphorylated form of tau. The conjugation-deficient point mutation, K340R, resulted in a loss of Tau SUMOylation and a decrease in phosphorylation [47]. It has been hypothesized that the microtubule-unbound tau species is the preferred target for SUMO conjugation. This is consistent with the observation that the major SUMO target, K340, is located within the fourth microtubule-binding residues and when tau is released from tubulin exposes this domain permitting the access of SUMOylation enzymes [50]. Accordingly, phosphatase inhibition by okadaic acid and colchicine-induced microtubule depolymerization markedly up-regulate tau SUMOylation, suggesting that once released from the microtubules, the soluble pool of tau is the preferred target [43].

The mechanism by which SUMOylation may affect phosphorylation of tau is not fully understood. It is possible that SUMOylation may alter the conformation of tau and make it a better substrate for protein kinases (including GSK-3β) [51–53]. Since SUMOylation and phosphorylation of tau are co-dependent and given the fact that hyperphosphorylation decreases tau solubility, it may be that SUMOylation contributes to the decrease of tau solubility and its subsequent degradation [47].

Tau is known to be degraded by the proteasome either through ubiquitin-independent [54,55] or ubiquitin-dependent process [30,48,49,56]. Tau contains several ubiquitination sites and NFTs in AD tissues are strongly immunoreactive for ubiquitin [57,58]. The disease-related consequences of tau ubiquitination have been subject of debate. Some studies indicate that tau is ubiquitinated as fibrillar lesions in AD brains [57–59], while others shows that soluble tau species are labeled with ubiquitin isolated from fresh AD brain tissue [60,61]. These observations lead to the conclusion that the ubiquitin system fails to target misfolded tau for proteolytic degradation. Recently it has been determined that the proteasome system does attempt to process tau during the early-to-intermediate stages of NFTs evolution. It was observed that ubiquitin labeling is a protective cell process that degrades tau before it becomes misfolded and aggregated. The formation of hyperphosphorylated tau also coincides with the early truncation of tau at aspartic acid 421 (D421) by caspases. This truncated variant of tau has been shown to aggregate at a higher rate than reported for the wild-type full-length tau [62]. Recently, the cytosolic Ubiquitin-C-terminal Hydrolase L1 (UCHL-1), which controls the physiological remodeling of synapses by modulating ubiquitin homeostasis, has been found to interact in an aberrant manner with the toxic 20–22 kD N-terminal tau fragment. This interaction critically contributes to the early dysfunction of synaptic mitochondria in AD pathobiology [63].

It is conceivable that there is a direct competition between tau ubiquitination and SUMOylation and that may alter the balance for these two post-translational modifications. Indeed, an interesting

dynamic interplay between tau SUMOylation and proteasome inhibition has been shown [43]. HEK293 cells co-expressing tau and SUMO1 treated with proteasome inhibitors significantly elevated the levels of monomeric tau as well as its ubiquitination. Consistent with these findings, the catabolism of the cytoplasmic pool of tau was reduced [30,48,49]. In agreement with this work, there is direct evidence that tau SUMOylation obstructs its ubiquitination in tau-overexpressing HEK293 cells. As a result, tau degradation was reduced whereas its aggregation was increased [47].

A progressive impairment of the ubiquitin-proteasome system occurring at late AD stages is at least partially responsible for the reduced degradation of tau proteins [64–66]. As a speculation, we could think that the increased protein SUMOylation in AD models [15] may be partially responsible for the dysfunction of ubiquitin-mediated tau degradation since the two PTMs compete for identical lysine residues. It is possible that multiple modifications occur since tau phosphorylation promotes its SUMOylation which competes for ubiquitination and the inhibited tau ubiquitination is therefore dependent upon its phosphorylation. A similar pathway has been already demonstrated for the ubiquitination of IkBa, known to be dependent upon its prior phosphorylation [67].

Such a direct competition between SUMO and ubiquitin for the conjugation of the same lysine residues appears thus realistic, especially considering that tau is ubiquitinated within the N-terminal microtubule-binding domains in which also the K340 SUMOylation site is contained [60,68]. Alternatively, it has also been proposed that the decrease in SUMO1 conjugation could be the result of mistrafficking of ubiquitinated tau to other subcellular compartments [43].

4. SUMOylation impact on α-synuclein

Synucleinopathies such as PD, Dementia with Lewy bodies (DLB) and Multiple System Atrophy (MSA) are a group of neurodegenerative disorders characterized by the accumulation of filamentous aggregates of α-synuclein as inclusion bodies within the cytoplasm [69]. Missense mutations and increased gene expression of α-synuclein are considered to form the basis of autosomal-dominant PD [70–72] and α-synuclein is the major component of the neuronal intracellular inclusions that define the histological hallmark of PD and DLB [73].

α-synuclein is an amyloidogenic protein belonging to the family of natively unfolded neuronal proteins similar to tau. It displays an extended conformation *in vitro* with little ordered secondary structure [28,74]. It is localized at presynaptic terminals [75] and implicated in synaptic vesicle trafficking, synaptic plasticity, inhibition of MAP kinase signaling and cell death potentiation under serum deprivation conditions [76–80]. Under physiological conditions, α-synuclein encodes an unstructured soluble protein but under pathological conditions it can undergo conformational transition from random coil to β-pleated sheet conformation that leads to fibrillization, formation of oligomers, insoluble aggregates and intracellular deposition [81–85]. This aggregation-prone protein is known to be the target of several post-translational modifications including phosphorylation [86,87], nitration [88,89], acetylation [90], ubiquitination [91,92], and SUMOylation [93,94]. These modifications affect both protein structure and function, modifying the ability of α-synuclein to form aggregates as well as its subcellular localization and function [69].

α-Synuclein purified from Lewy bodies is extensively phosphorylated on S129. Experiments performed on neuronal cell culture suggest that such modification strongly modulates interactions between α-synuclein and synphilin-1, leading to the formation of inclusions and possibly Lewy bodies in PD [81,86,95,96]. Nitration has also been found to promote the formation of

intracytoplasmic α-synuclein inclusions [89]; the ubiquitin E3 ligase Siah-1 has also been suggested to promote the mono- and di-ubiquitination of α-synuclein, again promoting its aggregation [92,97]. Recent investigations have demonstrated co-localization of α-synuclein deposits with SUMO proteins in the characteristic inclusions found in PD and MSA [93,98]. In a cell-free assay, in which recombinant proteins are added to react in conjugation conditions, α-synuclein was efficiently SUMOylated in the presence of an E3 ligase [94]. The SUMOylation of α-synuclein was also demonstrated in *in vitro* cell-based assays. For example, HEK293T cells were co-transfected with His6-tagged SUMO2 and α-synuclein, resulting in its SUMOylation and increased solubility [94]. No polySUMOylation or multiSUMOylation were detected [43]. Mono-SUMOylated α-synuclein has been suggested both in embryonic kidney cells and in fibroblast cells [99]. Insoluble α-synuclein aggregates were found to be SUMOylated both in neuronal cell cultures in the form of perinuclear aggresomes as well as in the halo of Lewy bodies in PD and DLB affected brains [93]. Endogenous α-synuclein was found efficiently conjugated with SUMO2 in transgenic mice, confirming that α-synuclein SUMOylation actually occurs also *in vivo* in the mouse brain under physiological circumstances [94].

In HEK293 cells, the SUMO E3 ligase hPc2 has been found to directly bind to α-synuclein promoting its covalent mono-SUMOylation, whereas other SUMO E3 ligases, such as PIAS1, PIAS3, and PIASy, do not have the same effect [99]. Moreover, the expression of the mono-SUMOylated α-synuclein increased in the presence of hPc2 in a dose-dependent way. Among the three SUMO isoforms, SUMO1 appeared to have the strongest effect on hPc2-mediated α-synuclein SUMOylation. This is consistent with the previous finding that SUMO1 is predominantly conjugated to the substrate via mono-SUMOylation, whereas SUMO2 and SUMO3 are capable to form polymer chains and generally are conjugated to their targets through poly-SUMOylation [100].

α-Synuclein contains two putative SUMO consensus motifs: one is a classical SUMO consensus acceptor site (K96: VK96KD) while the other one is a closely related motif (K102: GK102NE). In addition, 13 other lysine residues have been localized mainly within the core repeats. Genetic mutagenesis was used to examine SUMOylation at these sites by means of single point mutations replacing lysine with arginine. Co-expression of the K102R mutant α-synuclein resulted in a slight decrease in the level of SUMOylation as compared with wild type, suggesting that K102 may be a SUMO1 conjugation site. However, it may not be the only target residue. In parallel, data from another study suggested that α-synuclein ubiquitination and SUMOylation are independent processes and likely target different lysine residues [93]. Despite repeated attempts, the other putative SUMO site (K96) could not be directly investigated due to complications with antibody recognition. These two sites account for more than 50% of the α-synuclein SUMO conjugates; in fact simultaneous mutation of both lysines strongly impaired SUMOylation [43,94]. Furthermore, the mutagenesis of two acidic residues adjacent to the SUMO acceptor lysine was performed to test if SUMOylation was affected, given the fact that the consensus SUMO acceptor sites specifically require an acidic residue [101]. Indeed, the mutation of D98A and E104A impaired SUMOylation to a similar extent as the lysine-to-arginine mutations, further corroborating the hypothesis that K96 and K102 are the two major and specific α-synuclein SUMOylation sites [94].

Interestingly, in a recent report it was found that SUMOylation of α-synuclein abolishes its fibril formation in vitro. Particularly, 50% of SUMOylation was sufficient to completely block α-synuclein fibrillation *in vitro* and even a small proportion (10%) of SUMOylated α-synuclein substantially delays its fibril formation [94]. Notably, in a rodent PD model overexpression of mutant

K96R/K102R α-synuclein in dopaminergic substantia nigra pars compacta substantially exacerbated toxicity and the neurodegenerative effect [94]. It has also been shown that covalent conjugation of a single SUMO molecule to α-synuclein prevents pathognomonic fibril formation where there is also interplay between SUMOylation and phosphorylation [102]. It is therefore possible to speculate that SUMOylation deficiency potentiates α-synuclein neurotoxicity, as seen in yeast [102] and acts as a modulator of fibril/aggregate formation and maintaining the aggregation-prone α-synuclein in solution [94]. Other investigations found that a higher proportion of oligomeric α-synuclein species were SUMOylated *in vitro* but only upon proteasome inhibition [90,96]. One additional study found a potential role for SUMOylation of α-synuclein in targeting the protein to exosomes, showing that SUMO2 binding to α-synuclein facilitates membrane binding and release within extracellular vesicles. This raises the possibility that SUMOylation of α-synuclein may increase its release from cells and further the propagation of aggregates in neighboring cells [103]. Treatment of mammalian cells, including fibroblast cells, with the proteasome inhibitor MG-132 usually leads to the formation of stable and multi-ubiquitinated protein inclusions that can ultimately end up in cell death [104,105]. The effect of MG-132 on SUMOylation of α-synuclein is still controversial. In fact, if from one side the proteasome inhibition significantly increased α-synuclein levels, although SUMO modification of α-synuclein remained largely unaffected in response to MG132-mediated proteasome inhibition [43], from another side MG-132 treatment triggers α-synuclein SUMOylation, particularly hPc2-mediated, promoting the formation of intracellular aggregates [93,99].

In addition, it was shown that MG-132-induced α-synuclein aggregation increases cell viability in the presence of staurosporine, and this is mediated by increased α-synuclein SUMOylation. Experimental protocols in transfected COS-7 cells, pretreated with MG-132 and stimulated with staurosporine showed a cell protective effect in the conjugated α-synuclein/SUMO1 species, suggesting that increased α-synuclein aggregation, especially via increased SUMOylation, might function as a cytoprotector in the presence of toxic stimuli, such as staurosporine [99].

5. SUMO, Htt and polyglutamine disorders

HD together with Spinal and Bulbar Muscular Atrophy (SBMA), Dentato-Rubral and Pallido-Luysian Atrophy (DRPLA) and several forms of Spino-Cerebellar Ataxia (SCA), belongs to the group of CAG repeat polyglutamine diseases [106,107]. HD is a neurodegenerative disorder caused by the expansion of a CAG polyglutamine (polyQ) repeat within the gene coding for the Htt protein [108]. Htt appears to have a neuroprotective function and enhances production of neurotrophic factors, such as BDNF [109]. The “polyglutamine pathologies” are characterized by selective neuronal cell death in specific brain regions, including basal ganglia, cerebellum, brainstem and spinal motor nuclei [110]. Still quite controversial or uncertain are the hypothesis about the pathological mechanisms of HD and, more in general, of the other polyQ-diseases.

A growing body of evidence highlights that either a gain or a loss of function of Htt may contribute to the pathogenesis of HD. For instance, considering that Htt potentially behaves as a neuroprotector, promoting BDNF production, its loss-of-function has been proposed as pathognomonic of HD [109]. Alternatively, the blockade of the proteasome system has been hypothesized to be responsible for the accumulation of abnormal polyglutaminate proteins, like mutant Htt, that potentially became toxic [111]. The possible relationship between polyglutamine toxicity and the formation of characteristic polyglutamine aggregates and inclusions could represent

a key explanation for the development of the molecular marker for these pathologies. Accordingly, as soon as the threshold of the minimal polyglutamine repetition length is exceeded (generally in the range of 35 to 45 repeats), the mutant expanded proteins undergo conformational changes into a pleated sheet structure and aggregates in the cell nucleus or cytoplasm, forming typical "amyloid-like" inclusion bodies [112,113]. The neurons with aggregates are not necessarily the ones that mostly die. Polyglutamine aggregation and cell toxicity can be experimentally dissociated both *in vitro* and *in vivo*, leading to the conclusion that the relationship between aggregation and toxicity is not yet well defined [114].

Protein aggregation leading to inclusions formation is a complex biochemical process involving several steps of intermediate oligomeric species and small nascent fibrils known as "protofibrils." Such intermediates are the ones reported for Htt [112]. A partial unfolding or abnormal folding of the protein and, mainly, a specific proteolytic process appear to induce aggregation and accumulation. A number of studies have indeed suggested a role for proteolytic cleavage of polyglutamine proteins, since only antibodies to the N-terminus of Htt, but not antibodies to epitopes in the middle or at the C-terminus, were able to recognize the intranuclear inclusions in HD [115–117]. Several studies indicated that the most relevant fragments are generated by aspartic endopeptidases, caspases and calpains [118], forming the N-terminal stretches which become toxic when derived from the expanded polyglutamine repeat mutants [119]. Full-length Htt or the related pathogenic fragment (Httex1p 97QP) can be regulated by multiple and functionally relevant post-translational modifications, either by ubiquitination [120,121], acetylation, palmitoylation, phosphorylation or SUMOylation [122–124], which may influence each other.

Ubiquitination appears to reduce poly-Q toxicity, presumably by promoting the degradation of toxic proteins. Indeed, mutations in ubiquitin ligases enhance poly-Q toxicity in Drosophila, in mouse models and in striatal neurons [125–127], whereas overexpression of Parkin, an E3 ubiquitin ligase, can reduce poly-Q aggregation and suppress cytotoxicity [128]. Ubiquitination has also been proved to be a major factor for the inclusion formation [129]. An interesting interplay between ubiquitination and SUMOylation of Htt has lately emerged. The two PTMs compete for the same lysine residues in the amino-terminal domain, thus preventing proteins from degradation or altering their function and subcellular localization [130–132].

Treatment of HeLa cells (an *in vitro* system wherein SUMO modifications are highly active) with the proteasome inhibitor MG132 caused the accumulation of mutant high molecular weight Htt in the insoluble fraction, together with the accumulation of ubiquitin-modified cellular proteins. In contrast, soluble, monomeric Htt levels were maintained or slightly decreased, supporting the concept that impairment of proteasomal function increases levels of aggregating Htt [133]. Specifically, it appeared that the role of SUMO2 is fundamental since it caused a dose-dependent increase in insoluble Htt, comparable to the one observed with proteasome inhibition.

A proteomic analysis confirmed the importance of SUMO2 in the conjugation of aggregated proteins. HeLa cells treated with proteasome inhibitors resulted in the accumulation of conjugated forms of all SUMO paralogs in insoluble protein inclusions as well as in the accumulation on SUMO2 substrates of lysine-63-linked polyubiquitin chains. This finding suggests that SUMO modification of cellular proteins may represent a response to the presence of misfolded or oligomerized proteins (such as mutant Htt), and be involved in protein clearance mechanisms [134]. Another example of interaction between ubiquitin and SUMO in the HD pathogenesis has been investigated in Drosophila [108]. Expression of mutant Htt led to progressive degeneration, which

was reduced in flies heterozygous for SUMO and modestly worsened in flies with genetically reduced ubiquitination. Interestingly, the mutation of Htt in the target lysines (K6 and K9) substantially prevented degeneration. These data indicate that lysines residues are essential for degeneration and that SUMOylation at these sites results in a pathological response, inhibiting degradation [113]. However, the overall dynamic and cooperation between both modification systems may be more important than the conjugation of a sole modifier, separately.

In addition to Htt, a number of studies have investigated the related Ataxin family of Poly-Q proteins and the effects of SUMOylation on disease pathways. Ataxin-7 is linked to spinocerebellar ataxia type 7 (SCA7) which has been shown to be SUMOylated at a conserved lysine residue (K257). Modification by SUMO1/2 did not alter subcellular localization of Ataxin-7 but intranuclear inclusions that were not immunopositive for SUMO displayed increases in proteasome markers and ubiquitin as well as elevated caspase-3 [135]. Interestingly, mutation of the K257 SUMOylation site resulted in increased levels of SDS-insoluble aggregates and caspase-3 positive cells. In contrast, the SUMO-conjugated Ataxin-1 displayed increased aggregation and deposition that could be reduced by inhibitors of the JNK pathway [136]. Another ataxia disorder, Machado-Joseph Disease (MJD), results from the abnormal accumulation of Ataxin-3 which has can be SUMOylated at two different sites, lysine-166 (K66) and lysine-356 (K356) [137,138]. Modification of Ataxin-3 by SUMO1 results in an enhanced stability of the protein which results in increased toxicity as shown by elevated apoptosis in cell-based assays [137]. SUMOylation of Ataxin-3 at K356 results in decreased and also has functional consequences as demonstrated by a reduced p57 binding [138]. These findings suggest that SUMOylation may contribute to the functional regulation of Ataxin-3 and its role in ERAD pathways.

Similar to tau, an interplay between SUMOylation and phosphorylation has also been reported for PolyQ proteins. Phosphorylation at Ser13 and Ser16 effectively reduces mono-SUMOylation and increases poly-SUMOylation of Httex1p fragments with a highly expanded 97Q repeat [139]. It has been discovered that Httex1p poly-SUMOylation can be also increased by the stress-inducible kinase IKK, via phosphorylation of the previous mentioned serine residues Ser13 and Ser16 [139]. Oxidative stress and other cellular stressors that are known to influence protein SUMOylation are indeed also implicated in the HD pathology [140]. SUMO2 activity and expression has been demonstrated to be highly influenced by cellular stress [141], and actually it has been proved that both SUMO2 and SUMO1 are able to modify Httex1p [142].

The influence of SUMOylation on protein aggregation has recently become of high interest for Htt. It has been shown, for example, that the fusion of SUMO to the Httex1p N-terminus promoted its stabilization [113]. The Httex1p N-terminus has been identified as an important mediator of aggregation, localization in terms of cytoplasmic targeting, and protein stability [139,143–146]. The presence of SUMO modifications, therefore, could impair such activities, possibly masking that cytoplasmic retention signal and promoting the nuclear or subnuclear localization in some cell types more than in others, allowing for different levels of nuclear localization and selective neuronal toxicity [147]. Recent studies have indeed hypothesized that SUMOylation of Htt might alter the protein aggregation state and therefore affect HD pathogenesis.

In a cell-based assay, truncated Htt was discovered to be SUMO1 modified at K6 and K9. Specific mutagenesis of such target lysines (K6R and K9R) proved their role as primary conjugation sites in both striatal and HeLa cells [113]. Because the target lysines in Httex1p do not fall within a classic SUMO consensus sequence, a SUMO prediction software has also been used (SUMOplot,

Abgent) to support an *in vitro* SUMO modification assay and mass spectrometry analysis. The lysine residues K6 and K9 were again confirmed to be the major SUMOylation sites among the three N-terminal lysines (K6, K9 or K15) [142].

An interesting study has been conducted on post-mortem striatum (the region most profoundly affected in HD) from three control and three HD brains. Each of the HD patients displayed a remarkable accumulation of SUMO2-modified proteins in the insoluble fractions compared to controls. To a lesser extent, accumulation of SUMO1 was also observed, suggesting that these modifications could indeed be relevant *in vivo* and regulate the age-related pathogenic accumulation of mutant Htt during HD progression [142].

To confirm, truncated Htt fragment co-localized with His-tagged SUMO1 when transfected into immortalized striatal nerve cells in a cell-based assay [148], reflecting either direct modification of Htt by SUMO1 or co-localization of Htt with other SUMOylated proteins. In addition, the Htt peptide fused with SUMO markedly accumulated in neuronal models and, conversely, aggregates decreased when SUMOylation sites were eliminated. Moreover, the proline-rich region of Httex1p appeared to be essential for inclusion formation [113]. Even longer Htt fragments are SUMOylated; an initiating cleavage event has been found to occur at a caspase-6 cleavage site of Htt [149], creating a polypeptide of 586 amino acids (Htt586). Such fragment has also been discovered to be SUMO2 modified in the presence of a SUMO E3 ligase, and this modification is even enhanced when mimicking phosphorylation for both expanded and the unexpanded Htt586 [142].

Among the SUMOylation enzymes, PIAS1, a SUMO E3 ligase, has been elected as a potential candidate SUMO ligase for Htt [142]. Several known PIAS proteins have been proposed as E3 ligases [150] and suggested as possible therapeutic targets and modulators of SUMOylation. The Drosophila PIAS protein, Su(-var)2-10 (dPIAS) has been for instance studied due to its involvement on HD-like phenotypes [151]. Neuronal expression of the mutant expanded Httex1p (93Q) causes a progressive loss of visible rhabdomeres (photoreceptor neurons in the eye) [152], whereas a genetic reduction of dPIAS was neuroprotective, increasing the number of visible photoreceptor neurons. Accordingly, it was observed that a reduction of Drosophila SUMO (smt3) was protective in the same way [113]. PIAS1 was proven to be able to regulate the accumulation of insoluble Htt polypeptides in HeLa cells as well [142].

Finally, SUMO1 modification of mutant Htt was also associated with increased toxicity but decreased aggregation by the striatal enriched small guanine nucleotide-binding protein Rhes [153]. In agreement with these findings, it was observed that SUMOylation increases Htt accumulation but not the aggregate formation, resulting more toxic species than the unmodified one. Probably, SUMOylation increases the amount of toxic oligomeric species as well as the pathological capacity of mutant Htt to repress transcription [113].

6. SOD1 and ALS

ALS causes the selective loss of motor neurons leading to paralysis and ultimately death within 2–5 years. Most ALS cases are sporadic but autosomal dominant mutations in the gene encoding the copper-zinc superoxide dismutase (SOD1) are a major cause of familial ALS (fALS), generating approximately 20% of cases. To date, at least 119 different mutations have been identified, among which G93A and G85R are the most frequent [154,155]. Mutated SOD1 has cytotoxic properties, resulting from a gain-of-function [156–159].

The major fALS pathologic hallmark is aggregation of mutant SOD1 [160] which misfolds and aggregates to form proteinaceous intracellular inclusions, as reported in ALS patients, transgenic mice and in cultured cells [160–162]. The inclusions of mutant SOD1 also contain ubiquitin, proteasome components, molecular chaperones, neurofilament proteins and other proteins [162–164]. It has been demonstrated that SOD1 is SUMOylated and that this modification may regulate its degradation process [165,166].

Human SOD1 is a substrate of SUMO1 but not of SUMO2 or SUMO3. Moreover, by means of an *in-vitro* SUMOylation assay, it was confirmed that specifically SOD1 undergoes SUMO1 modification [165]. SUMOylation of SOD1 has been also confirmed by using co-transfected NSC34 cells, a motor neuron cell line generally employed for ALS pathomechanism studies [166,167]. Consistent with these observations, neither SUMO2 nor SUMO3 were found to cause significant modification of wild type SOD1 in NSC34 cells. In contrast, the mutant G93R and G85R SOD1 proteins were found to be modified by SUMO2 and SUMO3. Interestingly, only a limited amount of SOD1 proteins were SUMOylated, whereas the other proteins remained non-SUMOylated [166]. SOD1 K9 and K75 are two potential target for SUMOylation within the canonical consensus sequences LKGD and PKDE, identified using ‘‘SUMOplot prediction’’ (www.abgent.com/doc/sumoplot).

To investigate the conjugation site, mutagenesis studies were performed with substitution of these lysines with arginines. K75R mutations abrogate the SUMO1 modification of SOD1 [165,166] whereas discrepancies were found concerning K9R mutations. An *in vitro* assay demonstrated that lysine-9 (K9) is not SUMO conjugated [165], while an *in vivo* study reported that both K75R mutation and K9R significantly reduced SOD1 SUMOylation in motor neuron-like NSC34 cells and in HEK293 cells. However, SUMO2 and SUMO3 modification were observed mainly at K75 [166]. It is conceivable that the additional site at K9 is alternatively observed, due to differences between the *in-vitro* versus *in-vivo* assay systems.

Since aggregates formation is a major pathological hallmark in fALS, and also considering that SUMO modification has been shown to regulate the stability of proteins [130,132,168], SOD1 SUMOylation has been analyzed in order to evaluate its involvement in the pathological process. SUMO1 modification on K75 residue of SOD1 both enhances its stability (as evidenced by an increasing steady state level of the protein), as well as leads to its aggregation. SUMO1 overexpression indeed was found to support SOD1 aggregation and furthermore to co-localize into the aggregates. These results, however, were not replicated for SUMO2 and SUMO3, possibly indicating a role for the sole SUMO1 in the pathological process of fALS [165].

The aggregation of SOD1 mutants linked to fALS has been previously demonstrated occurring in CHO cells [169]. However, it was found that SUMO3 both promotes and accelerates the aggregation of fALS-linked mutant SOD1 proteins. It was shown in NSC43 cells that SUMO3 has a greater effect on aggregated formations than SUMO1. The K75R mutant but not K9R significantly reduced the rate of aggregation, confirming previous findings [166]. In addition, the non-SUMOylated mutant SOD1 co-immunoprecipitated with SUMO, whereas the non-SUMOylatable wild type SOD1, which do not form aggregates, was not detected in the SUMO immunoprecipitates. Considering that the majority of the SOD1 protein was found as non-SUMOylated, these findings suggest that the SUMO3 binding to mutant SOD1 accelerates protein aggregation, possibly by recruiting the large amount of non-SUMOylated SOD1 forms [166].

Finally, the different SUMO isoforms have been found to show preferential intracellular

localization. SUMO1 mainly localizes into the nuclear membrane whereas SUMO2/3 localizes in the cytosol [170,171]. Considering that SOD1 is a cytoplasmic protein, its association with SUMO2/3 rather than SUMO1 appears more feasible. Moreover, SUMO1 is mainly found in a conjugated form, whereas SUMO2/3 generally exists in a non-conjugated form *in vivo*, and its conjugation can be induced by several stimuli including heat-shock and oxidative stress [141]. Thus, given that SUMO2/3 modification is a major response to oxidative stress [172,173], and additionally considering that oxidative stress is a risk factor for the onset of aging-related neurodegenerative disorders including ALS, SCA and AD [174], it is plausible to hypothesize that the stress-induced elevation of SUMO3ylation accelerates the aggregation of mutant SOD1 in motor neurons and subsequently activates the cytotoxic pathways at the basis of ALS pathophysiology.

7. TDP-43 and SUMOylation pathways

TDP-43 is a 414 amino acid protein encoded by the *TARDBP* gene on chromosome 1. This multifunctional DNA- and RNA-binding protein is generally found in the nucleus where it is involved in many cellular functions, including RNA transcription, alternative RNA splicing, regulation of mRNA stability and microRNA processing [175–178]. Proteinaceous intracellular inclusions are common hallmarks of many neurodegenerative diseases and actually mutations of TDP-43 have been associated with ALS and frontotemporal lobar degeneration (FTLD) [179,180]. In these pathologies this aggregation-prone protein has been found to be a major constituent of the intracellular inclusions. Although the mechanism through which TDP-43 is involved in neuronal death and degeneration remains unclear, it is well documented that the *"TDP-43 proteinopathies"* [181,182] are characterized by tau- and α-synuclein-negative inclusion bodies, but formed by polyubiquitinated and hyperphosphorylated species of TDP-43 protein, both in its full-length or truncated (35 and 25 kD) form [183,184]. As with other protein misfolded neurodegenerative diseases, one hypothesis is that TDP-43 mediated toxicity has been linked to a toxic gain-of-function mechanism, as indeed suggested in two independent studies using yeast and human cell models [185,186]. The pathological insoluble aggregates are found largely in the cytoplasm of affected neurons rather than in TDP-43 classical nuclear location [187–189]. In addition, several recent studies documented that cytoplasmic TDP-43 aggregates co-localize with stress granule markers, i.e. cytoplasmic inclusions wherein RNA translation is selectively repressed during cellular stress [190]. In pathological conditions, TDP-43 is cleaved and redistributed to the cytoplasm where it forms insoluble and stable spherical oligomers that exhibit reduced DNA binding capability. They show neurotoxic activity both *in vitro* and *in vivo*, as found in the forebrain of transgenic TDP-43 mice and FTLD-TDP patients [191].

Interestingly, these oligomers share common epitopes with anti-amyloid oligomer-specific antibodies and in a cell free assay they have been shown to promote amyloid oligomer formation by cross-seeding of the AB peptide [191]. A similar structural conversion was already a well-known phenomenon in "prion pathology", wherein the misfolded scrapie PrP^{Sc} form can convert native PrP^{c} [192]. The prion-like spreading property has been investigated in brains with aggregated TDP-43 [193]. It has been postulated that this protein may spread the disease phenotype among neighboring neurons, thus playing a role in TDP-43 proteinopathy pathogenesis and possibly contributing to AD progression. Indeed, the C-terminal glycine-rich of TDP-43 shows sequence similarity to the prion protein [194] and C-terminal fragments of human TDP-43 (hTDP-43), showing the A315T mutation, are generally detected in *post mortem* specimens of ALS and

FTLD [195–197]. Furthermore, overexpression of both wild type and mutant hTDP-43 causes motor neuron degeneration in mice and rats [198–201] and transient expression of mutant hTDP-43 leads to spinal motor neurons apoptosis in chicken embryos [202].

TDP-43 primary transcript undergoes multiple alternative splicing variants both in mouse and human, and so far 10 of them (S1–S10) have been identified [175]. All but the non-spliced full-length TDP-43, the alternatively spliced variants, are expressed as truncated proteins lacking the C-terminal domain, site of the majority of missense mutations reported to be associated with sporadic and fALS cases [202–204]. The full-length human TDP-43 and a spliced isoform (TDP-S6), lacking the C-terminal, have been overexpressed in HEK-293 cells and mouse hippocampal neurons. Full-length TDP-43 was primarily found in the nucleus, as expected, whereas its shorter variant TDP-S6 showed mainly cytoplasmic and nuclear location, with extensive aggregation and highly insoluble inclusions [205].

Moreover, both detergent-soluble and -insoluble fractions displayed different degrees of PTMs for both TDP-43 and TDP-S6 variants including phosphorylation, proteolysis, protein polymerization but especially ubiquitination and ubiquitin-like modifications. In the same study [205], a multiplex stable isotope labeling with amino acids in culture (SILAC) strategy was used in combination with LC-MS/MS [206], in order to evaluate proteins that co-localize with the TDP-43 and TDP-S6 detergent-insoluble proteome [207].

By means of this quantitative proteomics approach, which metabolically introduces a mass difference into proteins by incorporation of heavy isotopic forms of arginine and lysine, a concomitant elevation in both ubiquitin and SUMO2/3 was found within the insoluble proteome after TDP-S6 and full-length TDP-43 overexpression. A novel interaction between SUMO2/3 and TDP-43 has been therefore proposed. Quantitative mass spectrometry further revealed K48 and K63 as target for ubiquitin modifications of the TDP insoluble fraction, indicating that the interplay of ubiquitination and SUMOylation might have an important role in TDP-43 regulation, although this requires further investigation.

The previous findings were confirmed via a deSUMOylation assay using SENP2 (decreasing SUMOylation levels were observed with increasing amount of SENP2). In HEK-293 cells expressing full-length TDP-43, the vast majority of endogenous SUMO2/3 was diffusely localized throughout the nucleus, whereas in TDP-S6-overexpressing cells, SUMO2/3 was specifically sequestered within TDP-S6 nuclear inclusions. Interestingly, SUMO2/3 were not found to co-localize with cytoplasmic aggregates of TDP-S6 whereas strong co-localization of endogenous ubiquitin with both cytoplasmic and nuclear TDP-S6 inclusions was instead observed. Notably, these data correlate with a localization in the nuclei but not in the cytoplasm for the SUMOylated TDP protein, moreover directly linking SUMOylation with TDP-43 insolubility [205]. Finally, although at present inhibition of TDP-43 aggregation is considered to be a major potential therapeutic avenue for ALS and FTLD, another study suggested that TDP-43 aggregation is only associated with the process of cell death but is not a direct primary cause [208].

In a recent investigation, HeLa cells overexpressing a mutant human TDP-43 were subjected to a high density peptide array analysis in order to identify candidate synthetic peptides possibly able to bind to TDP-43 and reduce its aggregation. Although aggregation levels were indeed reduced in a concentration-dependent way by this approach, the authors did not find any prevention or rescue from cell death, suggesting that reduction of TDP-43 aggregation does not necessarily prevent its

associated cytotoxicity [208]. As FTLD and ALS are neuron specific diseases, this study probably suffers from the limitations of a HeLa cell model chosen to perform the experiments.

8. Conclusions

SUMOylation is recognized as a fundamental intracellular mechanism involved in many cellular pathways. SUMO modifies the target proteins that can induce unique changes in their functions. It has been shown that SUMOylation plays a role in the regulation of transcription factors, cellular protein localization, receptor trafficking and many others. In this review, we have focused our attention on interesting and emerging features of protein SUMOylation that relate to its contributions to protein aggregation and degradation. There is considerable evidence indicating that protein SUMOylation takes part to the process of aggregation of several proteins that are intimately connected with the progression of neurodegenerative diseases. This is the case for proteins such Aβ, Tau, mutant Htt and mutant SOD1. In all these instances, SUMOylation enhances protein aggregation. As far it has reported SUMOylation of α-synuclein is able to increase its solubility and therefore prevent its aggregation. But still it is not clear if this is a protective mechanism or not (Figure 2).

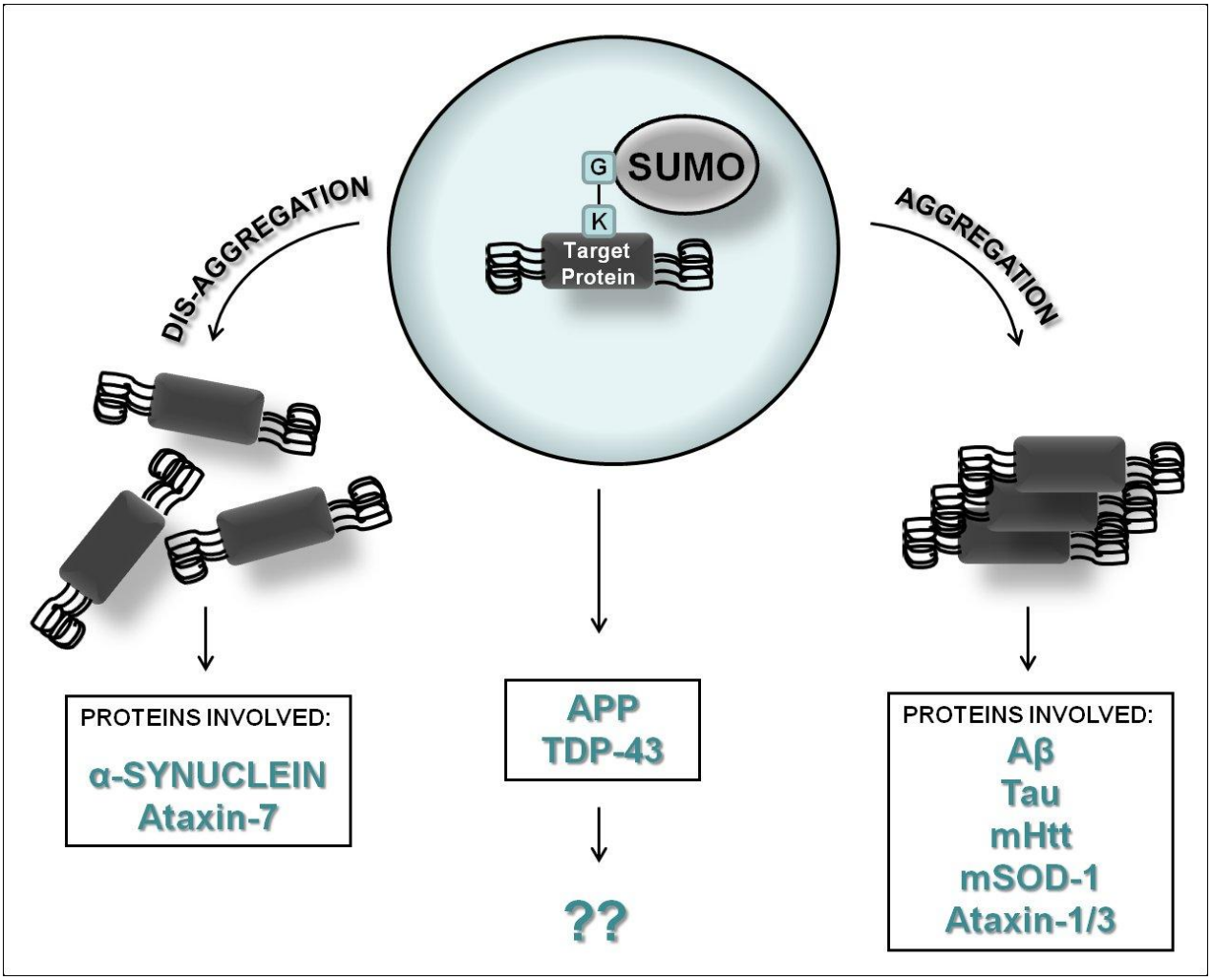

Figure 2. Protein aggregation is affected by SUMOylation. Protein SUMOylation has an effect on the aggregation of several proteins that are linked to pathologies. It is known

that SUMOylation of AB, Tau, mutant Htt, mutant SOD-1 and Ataxin-1 or 3 leads to an increase of protein aggregation, while it has been reported that SUMOylation of α-synuclein or Ataxin-7 reduces their aggregation. So far, the role of SUMOylation on APP cleavage is controversial and the role of SUMOylation on the aggregation state of TDP-43 is still unknown. It has also been shown that SUMOylation might also act at the downstream level of amyloidogenic proteins such as Aβ.

Moreover a protective role has already been observed for the aggregation process. Indeed, for example mutant Htt protein in HD is for instance toxic when soluble and its aggregation was shown to have no effect on cell survival [127]. Taking these considerations together a broader view arises, indicating that protein aggregation is not an obligatory factor leading to cytotoxicity and further investigation will be needed to deeply understand the molecular mechanisms at the basis of such a complicated concert. In conclusion, a significant number of studies support links to protein aggregation in a variety of neurodegenerative diseases and additional work in this area will allow us to better understand the mechanism by which SUMO contributes to the regulation of protein aggregation as well as the identification of new SUMO target proteins. SUMO also represents a potentially new therapeutic target in these neurodegenerative diseases, therefore a better knowledge on its involvement in protein aggregation could be important for future drug discovery focused on protein SUMOylation.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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